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Extraction of O-isobutyl S-(2-diethylaminoethyl)methyl phosphothioate (Russian VX) and related chemical signatures from hot dogs and analysis by Liquid Chromatography with Detection by Mass Spectrometry (LC-MS) and Gas Chromatography with Detection by Mass Spectrometry (GC-MS)

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Introductory Notes/Comments:

Approval, Concurrence or Review:	Name & Title/Position	Date
Approval Review Concurrence		
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1. SCOPE AND APPLICATION:

The following procedure describes the extraction of O-isobutyl S-(2-diethylaminoethyl)methyl phosphothioate (Russian VX) and related chemical signatures from hot dogs and analysis by Liquid Chromatography with Detection by Mass Spectrometry (LC-MS) and Gas Chromatography with Detection by Mass Spectrometry (GC-MS) to determine the Russian VX chemical attribution signatures present in a contaminated hot dog sample. These chemical attribution signatures may indicate the synthetic origin of the Russian VX sample.

2. SUMMARY OF METHOD:

A hot dog sample contaminated with Russian VX is processed using Solid Phase Extraction (SPE) to extract and preconcentrate the Russian VX and its related signatures from the hot dog matrix. The final extract is then analyzed with LC-MS and GC-MS to detect and identify the chemical attribution signatures.

3. INTERFERENCES:

3.1. Food Preservatives and Coloring agent – Paprika and salts

3.2. Food Flavorings – Sugars

4. SAFETY:

4.1. This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

4.2. Standard precautionary measures should be used for handling all solvents included in this method. Contaminated food samples should be treated as if they contain a high concentration of Russian VX until it is proven otherwise.

5. APPARATUS:

5.1. Thermo Scientific LTQ Orbitrap XL with a Surveyor Liquid Chromatograph and Autosampler.

5.2. Agilent 6890N gas chromatograph with a 5973N mass spectrometer detection system.

5.3. Leco Pegasus 4D GCxGC TOFMS system

5.4. Bella Rocket Blender (Sensio Inc., Montreal, Canada)

6. REAGENTS AND MATERIALS:

6.1. Waters Atlantis T3 RP (C18) Column, 250 mm x 4.6 mm, 5 μm particle size (Used for LC-MS analysis).

6.2. Agilent J&W DB-5MS (equivalent to (5%-Phenyl)-methylpolysiloxane), 30 m x 0.25 mm x 0.25 μm – used for GC-MS analysis).

6.3. Phenomenex Strata-X Solid Phase Extraction Cartridges, 33 μm, polymeric reverse phase (30 mg/3 mL).

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6.4. Syringes.
6.5. Methanol, LC-MS Grade.
6.6. Acetonitrile, LC-MS Grade.
6.7. Formic acid, ACS Grade.
6.8. Magnesium Sulfate, anhydrous, ACS grade.
6.9. Chloroform, ACS reagent.
6.10. Triethylamine, ACS reagent.
6.11. Water, Ultrapure, 18.2 MΩ.
6.12. Contaminated hot dog.
6.13. Uncontaminated hot dog (for process blank).

7. SAMPLE COLLECTION, PRESERVATION AND HANDLING:

Samples are collected and will be refrigerated until analysis.   Method development samples are homogenized and spiked with Russian VX in house. All handling and transportation will be in accordance with the LLNL chemical warfare agent safety procedures.

8. STANDARDS AND CONTROLS:

8.1. Russian VX (CV4021, BHOK8021, BHOK8043) (controls used in method development)
8.2. US d14-VX (HOK8079) (internal standard)
8.3. Triphenyl Phosphate, ≥99% (internal standard)

9. CALIBRATION:

9.1. Perfluorotributylamine (PFTBA) mass spectrometer tune solution.
9.2. ProteoMass LTQ/FT-Hybrid ESI positive mode calibration mix.

10. SAMPLING:

This method was developed using hot dog samples prepared by spiking in two (2) ways:
10.1. Commercially available hot dog spiked with Russian VX using a syringe.
    10.1.1. One hot dog link was segmented into 4 segments.
    10.1.2. The weight of each individual segment was noted.
    10.1.3. Agent was syringe injected into a segment to provide a spiked sample.
    10.1.4. Place an individual segment into blender and add ultrapure water equivalent to one half of known sample weight.
    10.1.5. Seal tightly and blend by pulsing in 10 second increments followed by gently tapping blender equaling two minutes of total processing time.
    10.1.6. Paste material was transferred into a clean glass jar and refrigerated.

10.2. Commercially available hot dog spiked with Russian VX after homogenization
    10.2.1. One hot dog link was weighted and placed into blender cup.
    10.2.2. Half of the weight of hot dog in ultrapure water was added.
    10.2.3. Sample blended by pulsing in 10 second increments followed by gently tapping blender equaling two minutes of total processing time.

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10.2.4. Paste material was transferred into a clean glass jar and refrigerated.

10.2.5. When needed, a known amount of agent was spiked onto a known amount of paste material.

10.2.6. Sample vortexed to thoroughly combine sample before extraction.

11. OTHER QUALITY ASSURANCE CONSIDERATIONS:

A reagent blank (methanol/0.003% formic acid) should be run with each set of samples, as well as a method blank, if an uncontaminated hot dog is available.   Samples should be extracted and analyzed in triplicate, if enough sample is obtained.

12. PROCEDURE (Step-by-Step Directions)

12.1. Sample Preparation

12.1.1. 5 grams of contaminated hot dog paste placed into 40 ml glass vial.

12.1.2. 10 mL water is added to vial.

12.1.3. 10 mL acetonitrile and 1 mL chloroform are added to vial.

12.1.4. 4 grams of magnesium sulfate, anhydrous, is added to vial.

12.1.5. Vial initially capped, shaken and evolved gases allowed to vent.

12.1.6. Vial vortexed for one minute.

12.1.7. Vial was centrifuged at 1500 rpm for 5 minutes.

12.1.8. Acetonitrile layer was transferred into a clean vial.

12.1.9. 10 µL of triethylamine was added to acetonitrile layer.

12.1.10. Sample was solvent exchanged using gentle stream of nitrogen gas to near dryness and dissolved into 3 mL of ultrapure water.

12.2. Solid Phase Extraction (SPE) procedure is as follows:

12.2.1. Condition – 2 mL methanol

12.2.2. Equilibrate – 2 mL ultrapure water

12.2.3. Load – 3 mL extracted sample

12.2.4. Wash - 2 mL ultrapure water

12.2.5. Elute

1 mL 0.01% formic acid in methanol.

2 mL methanol reagent.

12.3. Instrument conditions for LC-MS analysis are as follows:

12.3.1. Tune file

12.3.1.1.   Sheath gas flow rate: 15

12.3.1.2.   Auxiliary gas flow rate: 5

12.3.1.3.   Sweep gas flow rate: 5

12.3.1.4.   Spray Voltage: 3 kV

12.3.1.5.   Capillary temperature: 300 C

12.3.1.6.   Capillary voltage: 21

12.3.1.7.   Tube lens voltage: 70

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12.3.2. One scan event for 20 min

12.3.3. Scan range (m/z 85-1000)

12.3.4. Scan type: full

12.3.5. Polarity: negative

12.3.6. Column flow rate: 0.2 ml/min.

12.3.7. Column temperature: 30 °C.

12.3.8. Mobile Phase:

    12.3.8.1.   A= water + 0.1% formic acid

    12.3.8.2.   B= acetonitrile + 0.1% formic acid

    12.3.8.3.   100% A for 4 min

    12.3.8.4.   to 55% A at 5 min, hold 4 min

    12.3.8.5.   to 0% A at 10 min, hold 3 min

    12.3.8.6.   to 100% A at 14 min, hold 6 min

12.3.9. Injection volume: 10 μl.

12.3.10.   Diode array detector: on.

12.3.11.   NOTE: Allow system to equilibrate for approximately 20 minutes, or until a stable baseline is achieved.

12.4. Instrument conditions for GC-MS analysis are as follows:

    12.4.1. Carrier gas: Helium (UHP, 99.999% purity)

    12.4.2. Analysis mode: Constant Flow

    12.4.3. Column flow rate: 1.2 ml/min

    12.4.4. Injection volume: 1 μL.

    12.4.5. Injection type: Splitless

    12.4.6. Injector temperature: 250 °C.

    12.4.7. Oven temperature program:

        40 °C for 4 min

        8 °C/min to 300 °C

        300 °C for 1 min

    12.4.8. Mass analyzer: quadrupole, 150 °C

    12.4.9. Ionization: 70 eV electron ionization

    12.4.10.   Mass spectrometer: scan mode, m/z 29-500

    12.4.11.   Scan rate: 3.11 scans/s

    12.4.12.   Mass spectrometer source temperature: 230 °C

    12.4.13.   Solvent delay: 3 min

    12.4.14.   Mass spectrometer tune: standard, STUNE.u

12.5. Run each sample by aliquotting for direct injection:

    12.5.1. LC-MS sample can be directly injected.

    12.5.2. GC-MS sample can be directly injected.

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12.6. Data Analysis and Calculation of Recovery

Accuracy is estimated from the recovery of analytes from hot dogs. Laboratory performance is estimated from the recovery of analytes from a spiked process blank. Calculate the recovery (%R) of each analyte according to the following formula:

$$\%R = \frac{A_b}{A_s} \times 100$$

where:

A_b = Measured peak area in the hot dog sample.

A_s = Measured peak area in the spiked sample.

13. METHOD PERFORMANCE:

Performance data and related information are provided herein only as examples and guidance. The data do not represent required performance criteria for users of the methods.

14. REFERENCES

14.1. Anatassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J. (2003). Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce. J. of AOAC International. Vol. 86(2), 412-431.

14.2. Liu, G., Rong, L., Guo, B., Zhang, M., Li, S., Wu, Q., Chen, J., Chen, B., Shouzhuo, Y. (2011). Development of an improved method to extract pesticide residues in foods using acetonitrile with magnesium sulfate and chloroform. J. of Chromatography A. Vol. 1218. 1429-1436.

14.3. Wilkowska, A. Biziuk, M. (2011). Determination of pesticide residues in food matrices using the QuEChERS methodology. Food Chemistry. Vol. 125. 803-812.

15. POLLUTION PREVENTION

15.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

15.2. For information about pollution prevention that may be applicable to laboratories and research institutions consult Less is Better: Laboratory Chemical Management for Waste Reduction available from the American

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Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, http://www.acs.org.

16. POLLUTION PREVENTION

16.1. Waste contaminated with Russian VX should be neutralized before disposal. At LLNL, Document LLNL-MI-417220, "Neutralization Procedure for Chemical Agents and Toxins" should be followed. Decontamination will use commercial bleach to neutralize any agent remaining.

16.2. The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult The Waste Management Manual for Laboratory Personnel available from the American Chemical Society.